IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this PRELIMINARY AMENDMENT and the documents referred to as enclosed therein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service Mail Label No. EL587947765US under 37 CFR 1 10 on the date indicated below and is addressed to: Box Patent Application, Assistant Commissioner for Patents, Washington, D.C 20231.

Nancy L Barker — 9/25/01

Nancy L. Barker — Date of Deposit

Applicant: Sutcliffe, et al.

Serial No.: Unassigned

Filing Date: August 27, 2001

Title: METHOD FOR SIMULTANEOUS IDENTIFICATION OF DIFFERENTIALLY EXPRESSED mRNAs AND MEASUREMENT OF RELATIVE CONCENTRATIONS

Group Art Unit: Unassigned

Examiner: Unassigned

Our Ref.: TSRI 401.0 Div 5

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231
ATTN: BOX PATENT APPLICATION

Dear Sir:

Prior to the examination of the accompanying application on its merits, please amend the specification as follows:

IN THE SPECIFICATION

At page 1, line 7, please insert:

-- Reference to Related Applications

This application is a divisional of co-pending application serial No. 09/316,349, filed May 21, 1999 which is a divisional of application serial no. 09/035,190, which was filed March 5, 1998, and issued on February 29, 2000 as U.S. Pat. No. 6,030,784, which is a divisional of

application serial No. 08/544,577, which was filed October 17, 1995, and issued on September 15, 1998 as U.S. Pat. No. 5,807,680, which is a divisional of application serial No. 08/152,482, which was filed November 12, 1993, and issued on October 17, 1995 as U.S. Pat. No. 5,459, 037.--

At page 7, line 28, please substitute the following paragraph for the previous version:

It is unlikely that all mRNAs are amenable to detection by this method for the following reasons. For an mRNA to surface in such a survey, it must be prevalent enough to produce a signal on the autoradiograph and contain a sequence in its 3′ 500 nucleotides capable of serving as a site for mismatched primer binding and priming. The more prevalent an individual mRNA species, the more likely it would be to generate a product. Thus, prevalent species may give bands with many different arbitrary primers. Because this latter property would contain an unpredictable element of chance based on selection of the arbitrary primers, it would be difficult to approach closure by the arbitrary primer method. Also, for the information to be portable from one laboratory to another and reliable, the mismatched priming must be highly reproducible under different laboratory conditions using different PCR machines, with the resulting slight variation in reaction conditions. As the basis for mismatched priming is poorly understood, this is a drawback of building a database from data obtained by the Liang & Pardee differential display method.

At page 12, line 13, please insert

--Typically, in the present method the intensity of each band displayed after electrophoresis is about proportional to the abundance of the mRNA corresponding to the band in the original mixture. Typically the present method further comprises a step of determining the

relative abundance of each mRNA in the original mixture from the intensity of the band corresponding to that mRNA after electrophoresis.--

IN THE SEQUENCE LISTING

Please delete the SEQUENCE LISTING on pages 41-45 and replace with

-- SEQUENCE LISTING

<110> Sutcliffe, J. G.
Erlander, Mark G.

<120> Method for Simultaneous Identification of Differentially Expressed mRNAs and Measurement of Relative Concentrations

<130> TSRI 401.0D3

<140>
<141>

<150> US 08/152,482
<151> 1993-11-12

<150> US 08/544,577
<151> 1995-10-17

<150> US 09/035,190
<151> 1998-03-05

<160> 6

<170> PatentIn Ver. 2.0

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<211> 14
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<213> Artificial Sequence

<223> Description of Artificial Sequence:synthetic primer

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<213> Artificial Sequence

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<223> Description of Artificial Sequence:synthetic primer

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<223> Description of Artificial Sequence:synthetic primer

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<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence:synthetic primer

<400> 6
aggtcgacgg tatcggnnnn 20--

IN THE CLAIMS

Please cancel claims 1-37. Please add claims 38-43 as follows:

38. A method of producing a transformed polynucleotide sequence database entry, comprising the steps of:

choosing a source sequence from a polynucleotide sequence database entry;

locating a poly(A) tail sequence within the source sequence;

locating an endonuclease recognition site sequence within the source sequence that is closest to the first recognition site;

determining an index sequence consisting of about two to about six nucleotides adjacent to the endonuclease recognition site;

determining a correlate sequence within the source sequence, said correlate sequence including the sequence bounded by the poly(A) tail and the endonuclease recognition site and

including at least part of the endonuclease recognition site;

determining the length of the correlate sequence; and

storing information concerning the location and sequence of the poly(A) tail, the location and sequence of the endonuclease recognition site, and the length of the correlate sequence in relation to the source sequence, thereby producing a transformed database entry.

39. The method of claim 38 further comprising the step of:

displaying graphically the length of the correlate sequence in relation to the index sequence.

40. The method of claim 39 wherein the restriction endonuclease is chosen from the group consisting of MspI, TaqI and HinP1I.

41. A method of improving resolution of the length and amount of PCR products by diminishing background that is due to amplification of untargeted cDNAs comprising the steps of:

selecting a sample of a cRNA population, wherein each cRNA molecule comprises insert sequence and vector-derived sequence;

performing reverse transcription using a reverse transcription primer that hybridizes to the vector-derived sequence and that extends about five nucleotides to about six nucleotides into the insert sequence to produce a cDNA reverse transcription product;

subdividing the cDNA reverse transcription product;

performing at least one polymerase chain reaction using the subdivided cDNA reverse transcription product, a 3'PCR primer and a 5' PCR primer that hybridizes to the vector-derived sequence and extends about seven nucleotides to about nine nucleotides into the insert sequence to produce a PCR product, thereby diminishing background that is due to amplification of untargeted cDNAs.

42. The method of claim 41 wherein there are sixteen pools of reverse transcription reactions and there are 16 different reverse transcription primers.

43. The method of claim 42 wherein there are 4^x subpools of polymerase chain reactions, where X is the difference between the number of nucleotides that the 5' PCR primer extends into the insert sequence and the number of nucleotides that the reverse transcription primer extends into the insert sequence.

REMARKS

Entry of the amendments and examination in view of the amendments is respectfully requested. Based on the amendments and the accompanying discussion it is submitted that the claims as amended describe patentable subject matter, and therefore it is requested that the examiner consider the application in view of the amendments.

I. The Amendments

Many of the amendments are grammatical or serve to more particularly define the invention, and do not add new matter. The amendment to be inserted at page 1, line 7, sets forth the relationship of the present application to related applications. The amendment to be inserted at page 12, line 13 simply copies into the specification the text of claims 15 and 16 as originally filed, and as such adds no new matter.

The amendments to the Sequence Listing are to make the Sequence Listing consistent with the current requirements for form. No new matter is added.

Support for claim 38 is found at least at page 10, lines 8-33; page 17, line 28 to page 18, line 8; page 37, line 29 to page 38, line 3; page 40, line 13 to page 42, line 32; and Figures 1 and 2.

Support for claim 39 is found at least at page 40, line 13 to page 42, line 32; and Figures 1 and 2.

Support for claim 40 is found at least at page 11, lines 30-34; page 23, lines 9-14; and page 53, lines 9-12.

Support for claim 41 is found at least at page 28, line 4 to page 29, line 20 and page 32, lines 4-6.

Support for claim 42 is found at least at page 10, lines 8-33.

Support for claim 43 is found at least at page 30, lines 16-21; page 32, lines 1-21.

As can be seen from the support identified above, no new matter is added by the amendments.

II. Summary

Entry of the amendments is respectfully requested. The Examiner is invited to telephone the undersigned if it would be considered helpful in examining the application.

Respectfully submitted,

Dated: 9/25/01

By _____
Thomas Fitting, Reg. No. 34,163

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